



IN THE UNITED STATES PATENT OFFICE

Seulberger et al.

U.S. Application Serial No. : 09/462,629

Filed: January 11, 2000

For: „DNA sequence encoding a hydroxyphenylpyruvate dioxygenase, and its overproduction in plants“

DECLARATION

I, Ralf Michael Schmidt, Dr. rer. nat., a citizen of the Federal Republic of Germany and residing at D-67489 Kirrweiler, Am Schloßgarten 9d Federal Republic of Germany declare as follows:

I am a biochemist, having studied biochemistry in the period 1977 to 1986 at the University of Münster, Federal Republic of Germany.

I obtained my doctor's degree from the University of Münster in 1986.

I joined the BASF AG, Carl-Bosch str. 38, 67056 Ludwigshafen,

Federal Republic of Germany, in 1986.

Since 1994 I have been engaged in work in the field of plant biotechnology.

I am one of the inventors of the invention disclosed and claimed in Application Serial No. 09/462,629 and I am therefore familiar with the field to which the said application relates.

I intensively studied the Office Action mailed October 19, 2004 and know that the Examiner has rejected our claims 1 to 16 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement and does not reasonably provide enablement for an isolated DNA encoding any barley HPPD.

However, the person skilled in the art is able without any undue burden to isolate genes encoding for HPPDs from the genus *Hordeum* on the basis of the sequence information disclosed in the application at hand by using the state of the art techniques disclosed in the specification of said application (see specification page 7, lines 12 to 26 and examples 1 and 2).

These and other techniques were established and well known in the art at the priority date of said application.

To demonstrate the significant degree of identity between the different HPPDs sequences even if they do not belong to the same genus, we compared the HPPD encoding DNA sequence of the invention with a DNA sequence encoding the *Oryza sativa* HPPD using standard sequence comparison tools.

This comparison clearly demonstrates that these two sequences having a sequence identity of 84%.

Under consideration of the above given arguments, the identity between the claimed genes belonging to the genus Hordeum has to be expected to be at least 95%.

figure 1:

Sequence similarity table

H. vulgare HPPD	[100]	[84] [100]
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[identities in %]

H. vulgare HPPD	1	50
O. sativa HPPD	(1) ATGCCGGCCACCCCGAACGAAAGCCCGGGCTAACGGGCGCTG-----CGCG	
H. vulgare HPPD	(1) ATGCCGTCCCAGTCCCAGACCCCCAGCAGCAACACCGGCGGCGCTCTCGGGCGCG	100
O. sativa HPPD	(45) CGGGGTGACGCCGGAGCACGCCGGA-----CGCGA-----CGCAA	
H. vulgare HPPD	(51) TGCGGCCGGCGGGGAGACGCCGGGTTCCGCGCTCGTCGGGCACCGGCCGCT	150
O. sativa HPPD	(101) TGGTCCGCTTCACCCGGCAGCGACCGCTTCCACAGCGAGTGCGGTTCCAC	
H. vulgare HPPD	(101) TCGTCGGCGCAACCGGGAGCGACCCGGTTCCAGGGGCGGGGTTCCAC	200
O. sativa HPPD	(130) CGCGTCGAGTCTGGTCCGGGACGGCGCGCTCGCGCTCGCGCGCGCTTGCG	
H. vulgare HPPD	(151) CGCGTCGAGCTCTGGCTGGCGAGCGCGCTCGCGCGCGCGCGCGCGCG	250
O. sativa HPPD	(180) GTTGGCGCTCGGCCGCGAGCGCGCGAGGTCTGCGACCGCGCGCGCGCG	
H. vulgare HPPD	(201) CTTCGGCCCTGGCCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	300
O. sativa HPPD	(201) CTTCGGCCCTGGCCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	
H. vulgare HPPD	(230) ACTGGCGGGAGCGCTCCAGCTGGCTGGCTGGGAGTCGCGCTCGCGCTCG	
C. sativa HPPD	(251) ACTGGGGCACGCCCTCCCTCCCTCGCTCGCGCTCGCGCTCGCGCTCG	350
H. vulgare HPPD	(280) TTCAOCCGOGCCCTACGGCGA-----ACGGCTGGGAGCTCGCGCTCG	
O. sativa HPPD	(301) TTCAOCCGOGCCCTACGGCGGGGACCAACGGCGTGGGGGGGACCGCGGCCAG	400
H. vulgare HPPD	(315) GACCGCGCTCCCTCGCCCTCGCTCGCGCGAGGCCGGCGCGCGCGCGCG	
O. sativa HPPD	(351) GACCGCGCTCGATCCCTTCCTCTCGCGAGGCCGGCGCGCGCGCGCG	450
H. vulgare HPPD	(365) CGCAACCACGGGATSGGGGTGCGCTCGCTACGGCGTGTGCGCTCGCG	
O. sativa HPPD	(401) CGCAACCACGGGCTCGCGGTGCAACGGCGTGGCGCTCGCGACCGCG	500
H. vulgare HPPD	(415) CGCGAGGGCTTCGGCGCGCACTCGCGACGGGCGCGCGCGCGCGCG	
O. sativa HPPD	(451) CGCGAGCGCTTCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG	550
H. vulgare HPPD	(464) CGCGAGGGCTTCGGCGCGCGCTCGCGCTCGCGCGCGCGCGCGCG	
O. sativa HPPD	(500) AGCGCGCCGACCTCGCGGTGGCGCTCGCGCTCGCGAGGGAGGGAGCTAG	600
H. vulgare HPPD	(514) GCGCGAGGAGGGTGTCTCGCTTCGCTACGGCACCGCGCGCGCGCGCG	
O. sativa HPPD	(550) GGCGAGGTGCTGCTCCGCTTCGCTACGGCACCGGGAGGGCGCGCGCGCG	650
H. vulgare HPPD	(564) GTCCTTGCGGGGGTTCGAGGGCGTAACCAACCGGAGCGCGCTCGACTACG	
O. sativa HPPD	(600) GTCCTTCGGGGTTTCGAGGGCGCTACGGCACCGCGCGCGCGCGCGCG	700
H. vulgare HPPD	(614) GCGTGACGGGGTTCGACCGACGCTCGCTCGCGCAACGGCGCGCGCGCG	
O. sativa HPPD	(650) GCGTCGGCGGGTTTCGACCGACGCTCGCTCGCGCAACGGCGCGCGCGCG	750
H. vulgare HPPD	(664) GCGCGAGGGCTACATGGCGGGGTCACGGGGTTCGCTACGGCGCGCGCG	
O. sativa HPPD	(700) GTAGCGCGTACATCTCCGGTTCACGGGGTTCGCTACGGCGCGCGCG	800

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H. vulgare HPPD	(714)	GACGGGUGGAGGAACCTGGGCAAGACCGAGAGGGGGTGGATGCTGGTCC
O. sativa HPPD	(750)	CACCGCCGAGGGAACTGTGGGCAACCGGGAGAGGGGGCTGGATGCTGGTCC
		850
H. vulgare HPPD	(764)	TGGGGAACTGGTGGGGGCTGGTGGTGGCTGGCTGGGGGGGGGGGGGGGG
O. sativa HPPD	(800)	TGGGGAACTGGGGAGACCGTGGTGGTGGCTGGGGGGGGGGGGGGGGGG
		900
H. vulgare HPPD	(814)	GGGACCCAAGGCCGGAGCAGATTCAGAACGGTTGGTGGGGGGGGGGGG
O. sativa HPPD	(850)	GGGACCCAAGGCCGGGGAGCAGATTCAGAACGGTTGGTGGGGGGGGGG
		950
H. vulgare HPPD	(864)	GGGGGGCTGGACCAAGTGGGGCTGGGCGAGGGTGGTGGGGGGGGGGGG
O. sativa HPPD	(900)	GGGGGGGGCTGGACCAAGTGGGGCTGGGCGAGGGTGGTGGGGGGGGGG
		1000
H. vulgare HPPD	(914)	TCAAGGAAGATGGTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
O. sativa HPPD	(950)	TGAGGGAGATGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
		1050
H. vulgare HPPD	(964)	CCCCCGGTGGCGAACTACTAACGAAAGGGGTGGGAGCCCTTGCGGGGGATGT
O. sativa HPPD	(1000)	CCGGCCCCCCCCAACCTACTAACGAAAGGGGTGGGAGCCCTTGCGGGGGACGT
		1100
H. vulgare HPPD	(1014)	CCCTTCGGGAGGGGGAGATTCAGGAATGGGAGGGGGGGGGGGGGGGGG
O. sativa HPPD	(1050)	GCCTTCGGGAGGGGGAGATTCAGGAATGGGAGGGGGGGGGGGGGGGGG
		1150
H. vulgare HPPD	(1064)	ATAAGGGACGACCAAGGGGTGGTGGTGGAAATCTTGCGGGGGGGGGGG
O. sativa HPPD	(1100)	ACACGGGATGATGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
		1200
H. vulgare HPPD	(1114)	GACAGGGGGAGCTTGTTGGTGGAGATGATTCAGGGATCGGGGGCATGGG
O. sativa HPPD	(1150)	GCACGGGGAGCTTGTTGGTGGAGATGATTCAGGGATCGGGGGCATGGG
		1250
H. vulgare HPPD	(1164)	GAACGGACGAGAGGGCAAGAGTACCCAGAAGGGTGGGGGGGGGGGGGG
O. sativa HPPD	(1200)	GAACGGATGAGAGTGGGGCAAGAGTACCCAGAAGGGGGGGGGGGGGGG
		1300
H. vulgare HPPD	(1214)	GCACAAAGGGAACTTCTCCGAGGTGGTGGTGGTGGTGGTGGTGGTGG
O. sativa HPPD	(1250)	GGAAAGGGGAACTTCTCCGAGGTGGTGGTGGTGGTGGTGGTGGTGGTGG
		1342
H. vulgare HPPD	(1264)	TCCCTTGAGGCAAGCAATCTGGTGGTGGTGGGGATGA---
O. sativa HPPD	(1300)	TCCCTTGAGGCAAGCAAGGGGGTACACTTCAAGGAATGCTAG

Higgins, D., J. Thompson, T. Gibson, J. D. Thompson, D. G. Higgins, Tl J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Research 22:4673-4680.

I further declare that all Statements made herein of my own knowledge are true and that Statements made on information or belief are believed to be true; and further that these Statements are made with the knowledge that willful false Statements and the like so made are punishable by fine or imprisonment , or both, under Section 1001 of Title 18 of the United States Code and that such willful false Statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at 67056 Ludwigshafen, Germany, December 15, 2004



signature of declarant